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REVIEW

Nano-Proteolysis Targeting Chimeras (Nano-PROTACs) in Cancer Therapy

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Abstract: Proteolysis-targeting chimeras (PROTACs) are heterobifunctional molecules that have the capability to induce specific protein degradation. While playing a revolutionary role in effectively degrading the protein of interest (POI), PROTACs encounter certain limitations that impede their clinical translation. These limitations encompass off-target effects, inadequate cell membrane permeability, and the hook effect. The advent of nanotechnology presents a promising avenue to surmount the challenges associated with conventional PROTACs. The utilization of nano-proteolysis targeting chimeras (nano-PROTACs) holds the potential to enhance specific tissue accumulation, augment membrane permeability, and enable controlled release. Consequently, this approach has the capacity to significantly enhance the controllable degradation of target proteins. Additionally, they enable a synergistic effect by combining with other therapeutic strategies. This review comprehensively summarizes the structural basis, advantages, and limitations of PROTACs. Furthermore, it highlights the latest advancements in nanosystems engineered for delivering PROTACs, as well as the development of nano-sized PROTACs employing nanocarriers as linkers. Moreover, it delves into the underlying principles of nanotechnology tailored specifically for PROTACs, alongside the current prospects of clinical research. In conclusion, the integration of nanotechnology into PROTACs harbors vast potential in enhancing the anti-tumor treatment response and expediting clinical translation.

Keywords: Proteolysis-targeting chimeras, nanotechnology, delivery, linker, designing principles

Introduction

Proteolysis-Targeting Chimeras (PROTAC)

Proteolysis-targeting chimeras (PROTAC) are a class of small molecule drugs designed to target and degrade intracellular proteins.[1] PROTAC consists of three parts; E3 ubiquitin ligase ligand, target protein ligand, and linker for conjugation. Due to the proper hydrophile-lipophile balance, PROTAC may be rapidly internalized into the cytoplasm, facilitating effective degradation of the protein of interest (POI). The target protein ligand and E3 ubiquitin ligase ligand guide PROTAC to recognize the target protein and E3 ubiquitin ligase, respectively, resulting in the formation of a ternary complex. As E3 ubiquitin ligase is an enzyme that can transfer ubiquitin molecules to a lysine of the target protein, the target protein turns to proteins marked with ubiquitin. The ubiquitin-proteasome systems rapidly and irreversibly initiate the degradation process, contributing to reduced levels of target proteins.[2–5] The schematic route of PROTAC is illustrated in Figure 1.

PROTAC offers several advantages over traditional small molecule inhibitors (SMIs), as described below, and puts it ahead of alternative drugs: 1) Changing the target from "undruggable" to "druggable".[6–10] "Undruggable" proteins refer to therapeutic targets with clinical significance that are characterized by the lack of definite ligand binding pockets, non-catalytic interaction regions, and unresolved crystal structures, accounting for 80% of proteins in human cells. Antibodies with high molecular weights cannot enter because the majority are located in cells or nuclei. Moreover, the protein

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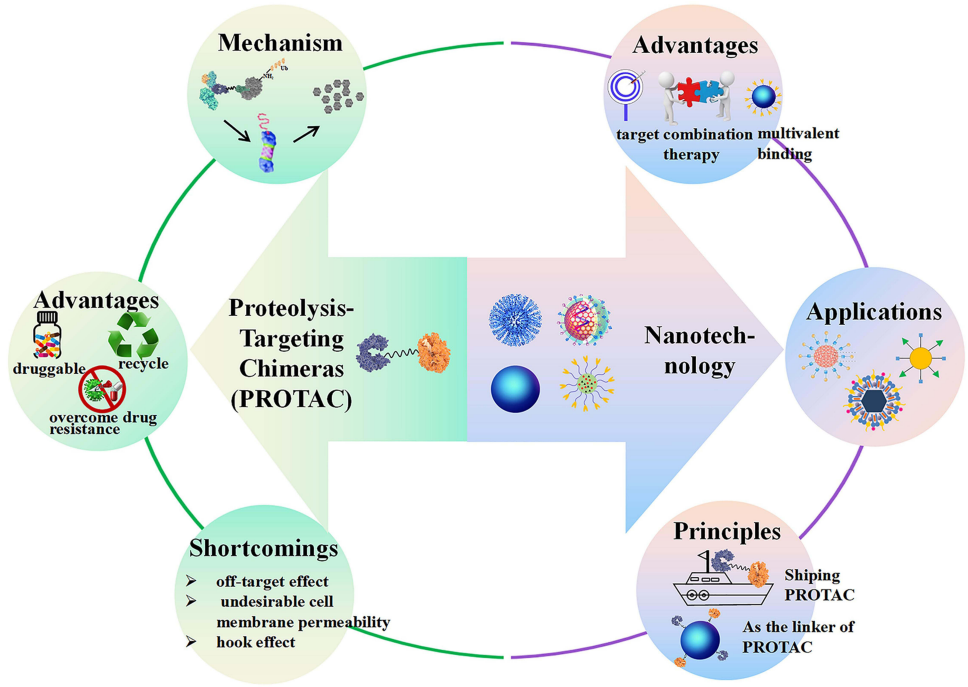
Graphical Abstract



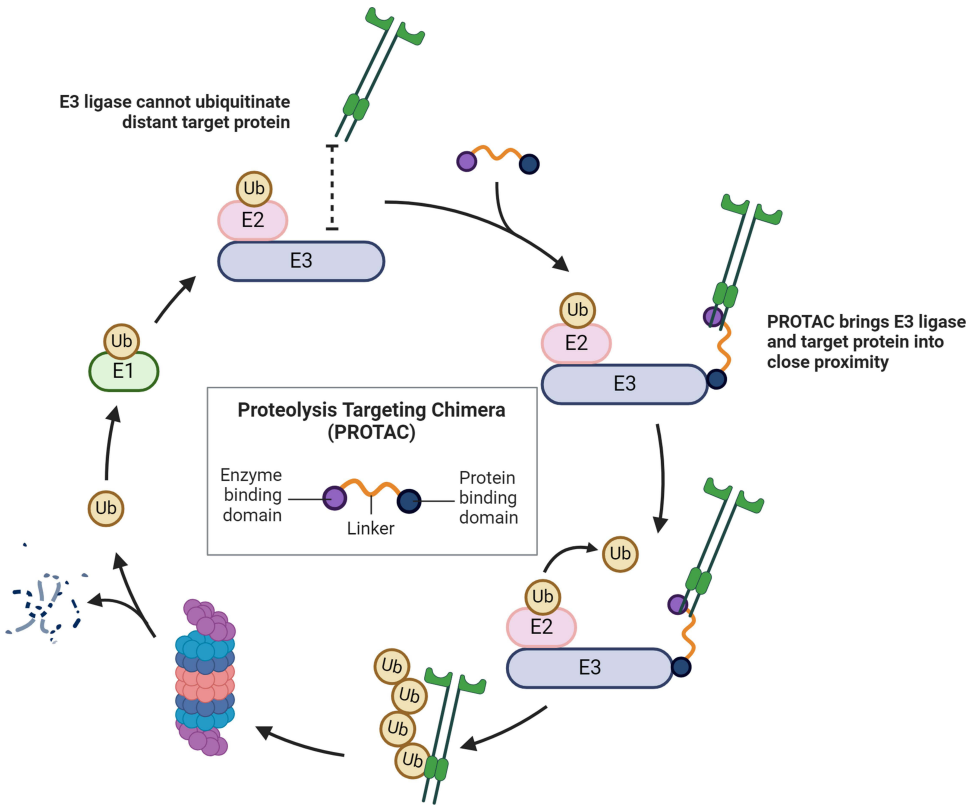


Figure 1 The mechanism of PROTACs. Figure generated using BioRender, Agreement number: KE26PEXHAO.

surface is relatively smooth without evident "pockets", thus, SMIs cannot be firmly grasped. Hence, these target proteins (including C-Myc, NF-κB, and brachyury (TBXTA)) are difficult or impossible to make into drugs via traditional methods. Conversely, PROTACs only require weak binding with the target protein to specifically "label" it regardless of catalytic ability or binding pockets of an enzyme. Currently, approximately 80% of the "undruggable" proteins may be solved by PROTAC technology.[11,12] 2) Overcoming drug resistance caused by mutation/overexpression of target proteins.[13–18] Over the course of the clinical use of SMIs or antagonists, acquired drug resistance will inevitably occur. For example, epidermal growth factor receptor (EGFR)-T790M[19] and C797S[20,21] are resistant. Even if the problem of drug resistance can be solved by creating new inhibitor generations, like the third and fourth generation of EGFR, using these generations of medications will lead to new drug resistance. The use of PROTAC technology has been beneficial in combating medication resistance. Recently, a C4 therapeutics company announced its EGFR degrading agent CFT8919, making the fourth-generation inhibitor currently under development inferior. CFT8919 exhibits strong degradation activity to a variety of EGFR mutants, including the C797S drug-resistant mutation that is currently unavailable in clinics, and can avoid the degradation of the EGFR wild-type. The transplanted tumor model in vivo further verified that the degradation agent exhibited good tumor inhibitory activity and had little effect on body weight; indicating its safety.[22] Although the specific structure of CFT8919 has not been disclosed, through the analysis of relevant patents we can determine that the connected ligand binding to EGFR is based on allosteric inhibitors and the connected ligand of E3 ligase is based on CRBN. 3) Recycling mode.[23] After one round of degradation, PROTAC will be released, which enables the PROTAC to be reused. Therefore, theoretically, the dosage of PROTAC can be efficiently reduced while achieving the equivalent therapeutic outcomes. 4) Tissue-and/or tumor-specific E3 ligase ligands can be used to achieve tumor selectivity.[24] Selective degradation of tumor-associated proteins of interest (POIs) can be achieved using PROTACs through various strategies. When the POI is specific to the tumor, tumor-specific PROTACs can be generated by directing the POI towards any available E3 ligase present in the tumor tissue. For instance, Crews et al utilized von-Hippel Lindau (VHL) ligands to create PROTACs that target the oncogenic fusion protein BCR-ABL1 in chronic myeloid leukemia (CML) cells, which are exclusively expressed in CML cells.[25] Additionally, tumor-selective PROTACs can be generated by directing the POI towards any available E3 ligase in tumor-derived tissue, even if the POIs are not specific to the tumor but functionally unnecessary in normal tissue. Several PROTACs have been developed to target BTK, a protein crucial for normal B cell development and B cell lymphoma progression, showing promise in treating ibrutinib-resistant non-Hodgkin lymphoma.[26] In cases where the POI is not specific to a particular tissue or tumor, targeting tissue- or tumor-specific E3 ligases can generate tumor-selective PROTACs, thereby reducing the toxicity of the POI. An example of this strategy is DT2216, a BCL-XL PROTAC that directs BCL-XL toward the VHL E3 ligase for degradation.[27] Platelets rely on BCL-XL for survival, and the use of dual BCL-XL/BCL-2 inhibitors like ABT263 or selective BCL-XL inhibitors (A1155463 or A1331852) can lead to severe platelet toxicity, limiting their potential as safer anticancer therapies. Researchers have employed PROTAC technology to selectively target anti-apoptotic BCL-XL proteins in tumor cells by utilizing the least expressed VHL or CRBN E3 ligases in platelets, thereby reducing platelet toxicity.

Despite these distinct advantages, the development of PROTACs is still limited in terms of clinical translation. 1) Off-target effect.[28–30] A lack of tissue-targeting ability when PROTACs are administrated orally or intravenously may produce off-target effects on extra organs, causing adverse effects. 2) Cell membrane permeability.[31] PROTACs need cell membrane penetration to be effective, as reflected by an appropriate hydrophile-lipophile balance in the components. Moreover, a linker of appropriate length is required, which does not bind to two target proteins due to too the close distance or ubiquitinate target proteins due to the far distance. Therefore, constant optimization of the chemical structure of PROTACs has been performed, which is time-consuming and causes a high workload and economic burden. 3) Hook effect.[32] Even though the obtained PROTACs can pass through cell membranes, the amount of target proteins and PROTACs are still not equivalent. This may be attributable to the inappropriate concentration and affinity between supply and demand. Thus, the development of PROTACs is still a long way from completion. Additional methods should be initiated to improve therapeutic response and advance clinical translations of PROTACs.

Discovery of Nano-PROTACs

The emergence of nanotechnology forms the foundation for the clinical translation of PROTACs due to its intrinsic physicochemical properties. Nanotechnology commonly refers to nanoscale structures up to 200 nm in size. Nanoplatforms designed for drug delivery include liposomes,[33–37] solid nanoparticles,[38–40] nanoparticles,[41–43] polymeric micelles,[44,45] nanogels,[46–48] and extracellular vesicles.[49–51] Agents can be loaded into the core or on the surface of nanosystems via chemical conjugation, physical encapsulation, or electrostatic adsorption. This nanosized process will endow drugs with an extended blood circulation time,[52] enhanced bioavailability,[53] improved therapeutic response,[54] and reduced side effects.[55] Over the last few decades, the advantages of nano drug delivery systems (NDDS) have been powerfully explored for a variety of treatments. To date, over 30 nanosystems types have entered clinical trials.[56] A randomized study concerning liposomal vitamin C in healthy, adult, human subjects revealed that, compared with non-liposomal encapsulated vitamin C,[57] liposomal preparations represented enhanced bioavailability accompanied by uniform particle size. In parallel, a study on amikacin liposome inhalation suspension demonstrated that this type of nanoscale particle exerted a potential benefit in patients with *Mycobacterium avium* complex lung disease.[58–60] Nanotechnology offers great advantages for tumor treatments. Pegylated liposomal doxorubicin, at a tolerated dose of 40 mg/m^2 every four weeks without serious adverse effects, is effective as a first-line treatment for platinum-resistant or -refractory recurrent ovarian cancer, according to a meta-analysis.[61] Patients who had severe tumors in solid tissue had another liposomal mimic of microRNA-34a (miR-34a) identified and assessed.[62] Findings showed modest therapeutic effectiveness and a reasonable toxicity profile in the majority of patients, demonstrating the viability of miRNA-based cancer therapy.

In general, nanodevices manifested three key advantages that may be beneficial for PROTAC delivery.

1. Improvement in drug concentrations at a lesion. It was reported fenestrations occurred in the tumor vascular wall which were not closely arranged and permeable. Further, the internal lymphatic drainage of the tumor was insufficient. When nanoparticles penetrate the tumor's interstitium, they stay there. This phenomenon refers to the enhanced permeability and retention (EPR) effect. Moreover, the possibility of nanoparticles entering the tumor site can be further improved by modification (ligands,[63] oligonucleotides,[64] antibodies, and cell membranes) on the surface of nanoparticles. Thus, the application of nanotechnology may contribute to improved drug concentration in tumor tissues.
2. High specific surface area and multivalent binding possibility. There are many modification sites on the nanosized surface of NDDS which may realize multivalent binding and increase the probability of binding target proteins.
3. Combination therapy in one location. Nano-architecture materials provided sufficient space to carry a variety of drugs to achieve synergistic therapy in one system.
4. Spatiotemporally-controlled drug release. To avoid off-target effects, PROTACs need to be released in the specific target cells to ensure proper function. With appropriate design, some NDDSs could function in response to temperature/light/magnetic fields locally, contributing to precisely controlled drug release based on requirements.

In this review, we introduce the current applications of nanotechnology for PROTACs, including nano-sized PROTACs for single or combination therapy for solid tumors, and NDDS designed for delivering PROTACs to tumor tissues (Table 1). Finally, we provide a future outlook on PROTAC-based nanoplatforms for tumor therapy.

Nanotechnology for PROTAC

Delivering Existing PROTACs

Single Tumor Treatment

Murine double minute X (MDMX) can induce p53 binding to non-specific chromatin regions while preventing p53 binding to specific target promoters, hence severely inhibiting the tumor suppressor gene p53. Therefore, lowering the amount of MDMX in cancer cells may improve the result of p53-mediated anti-tumor activity.[75] Thus, a novel concept for MDMX inhibition will be offered by peptide-derived protein degradation. Based on this, Yan et al[66] created a peptide-based PROTAC for MDMX degradation that includes the target recruitment component in the Cul2-Rbx1-EloB/C-VHL

Table 1 Application of Nanotechnology for Proteolysis-Targeting Chimeras

Composition	Size	Zeta Potential	Degraded Protein	Tumor Type	In vivo Anti-Tumor Effect	In vitro Anti-Tumor Effect	Reference
Cancer cell membrane (CCM) coated mesoporous silica nanoparticles (MSNs) containing paclitaxel (Pac) in its pores, linked to a peptide-based BMI1 PROTAC drug via disulfide bonds (PepM@PacC); the second were R837-loaded CaCO3("RC") nanoparticles.	PepM@PacC 210nm; RC 35nm;	/	The polycomb ring finger oncogene BMI1	Head and neck squamous cell carcinoma (HNSCC)	After PP-RC-H treatment was able to strengthen immunotherapy, promote BMI1 degradation, eliminate SCC7 tumor-bearing mice primary tumor, and effectively inhibit tumor metastasis.	Macrophages polarized from M2 to M1 kinds as a result of RC. M1 macrophages rise from less than 30% to more than 70%, while the percentage of M2 macrophages falls from 70% to less than 50%.	[65]
Semiconducting polymer nano-PROTAC(SPN_{pro}), which conjugated with the proteolysis targeting chimera via a Cathepsin B cleavable linker. The proteolysis targeting chimera includes an indoleamine 2,3-dioxygenase inhibitor and an E3 ligase-binding peptide.	28 nm;	/	Indoleamine 2,3-dioxygenase, IDO	4T1 tumor	The distal tumors were significantly suppressed, and the primary tumor was simultaneously completely suppressed.	At 40 μg/mL PCB, the survival of 4T1 cells was only 10% of that of the control.	[29]
Nano-MP@PSI is the synthesis of nanoengineered gold (I) -peptide clusters (Nano-MP) by self-assembly between affinity-driven gold peptide preforms [Au(I)-S-MP]n, coated with a ph-responsive polyacryl sulfydryl imidazole (PSI).	Nano-MP @PSI 28.3nm	Nano-MP @PSI 28mV	MDMX (MDM4)	Retinoblastoma and PDX model of pancreatic carcinoma	The ubiquitination-dependent degradation of MDMX was induced by Nano-MP@PSI, which also demonstrated in vitro activity of the p53 and p73 pathways.	Nano-MP @ PSI had enhanced therapeutic effects on KRAS mutant pancreatic carcinoma and retinoblastoma through degradation of MDMX and subsequent p53 and p73 restoration while maintaining a high degree of favorable biological safety.	[66]
Cer/Pom-PEG@GNPs are pegylated gold nanoparticles loaded with both pomalidomide and ceritinib molecules.	172nm	+12.1 mV	Anaplastic lymphoma kinase (ALK)	NCI-H2228 cell line	When ceritinib was used to compute the concentration of 4 μM Cer/Pom PEG@GNP, more than 80% of ALK fusion proteins were destroyed.	/	[67]

(*Continued*)

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Table 1 (Continued).

Composition	Size	Zeta Potential	Degraded Protein	Tumor Type	In vivo Anti-Tumor Effect	In vitro Anti-Tumor Effect	Reference
CREATE (CRV-LLC membrane / DS-PLGA/dBET6), which loaded BRD4-targeted PROTAC (dBET6) via DS-PLGA and then camouflaged it with engineered lung cancer cell membranes	229.71 ± 72.1 nm	−29 mV	The epigenetic reader bromodomain-containing protein 4 (BRD4)	The orthotopic and subcutaneous LLC tumor	CREATE simultaneously targeted TAM cell apoptosis in cancer cells and their surroundings, in part in a caspase −3 dependent manner.	In the LLC tumor-bearing mice, tumors in the CREATE treatment group were 1/30 the size of those in the saline group. In an in-situ LLC tumor model, about 90% of mice survived longer than 18 days.	[68]
(NPRO) included photosensitizer (protoporphyrin IX, PpIX), caspase 3 lysable peptide substrate (DEVD), UBR E3 ligase targeting fragment (RLAA), and the SHP2-targeting peptide (SP, SLNpYIDLDLVK).	40nm	−10mV	The Src homology 2 domain-containing phosphatase 2 (SHP2)	4T1 tumor	The caspase 3 linked with apoptosis may activate NPRO and cause SHP2 breakdown.	The anti-tumor immune responses were successfully enhanced by NPRO-mediated photoimmunotherapy, which induced ICDs in tumor cells and reprogrammed immunosuppressive checkpoint signaling pathways (PD-1/PD-L1 and CD47/Sirp-α) by degrading SHP2.	[69]
SPN_{COX} comprised a semi-conducting polymer foundation connected to COX-1/2-targeting PROTAC brushes via cancer-specific biomarker (Cathepsin B)-cleavable peptides.	30nm	/	COX-1/2	4T1 tumor	In comparison to control cells, the MFIs of the anti-COX-1/2antibodies in SPN_{COX}-incubated cells were much lower, falling by 92.0% for COX-1 and 89.1% for COX-2.	Mice injected with SPN_{COX} after NIR irradiation showed a decrease in immune suppressor cells (Tregs, M2 Macs, and MDSCs) and an increase in immune effector cells (Teffs, granzyme B+ Teffs, and Tems). This improved tumor immunogenicity effectively inhibited the tumor's growth, metastasis, and recurrence.	[70]
The PEGylated nanoliposomes (ARNIPL) contained BRD 4 proteolytic targeting chimeras (ARV-825) and nintedanib.	111.1 ± 6.55nm	+13.9 ±6.62mV	BRD4	Melanoma	The apoptosis induced by ARNIPL treatment.	ARNIPL treatment reduced tumor volume by 71.43% compared with control.	[71]

CM8988-PIPD: A PROTAC induced PDEδ degrader (PIPD) camouflaged with cancer cytomembrane.	124.8 nm	−7.59 mA	PDEδ	Pancreatic carcinoma	CM8988-PIPD had the highest apoptosis rate in all groups (53.7% in PATU-8988 cells and 54.3% in PL-45 cells), and could effectively induce PDEδ degradation in both concentration and time dependence.	/	[72]
A nanotherapy platform (T-ARV) based on TaTe2 nanosheets loaded with ARV-825.	/	/	BRD4 and C-MYC	Oral squamous cell carcinoma	T-ARV photothermal converted to 62.3% and briefly degraded BRD 4 / C-MYC at low concentrations.	T-ARV enables transient multimodal treatment of tumors under NIR irra diation in vitro.	[73]
dGPX4 is firstly prepared by the conjugation of GPX4 inhibitor (ML162) with pomalidomide. Then dGPX4 is encapsulated in lipid nanoparticles composed of reactive oxygen species (ROS) - degradable lipid 401-TK-12.	/	/	Glutathione peroxidase 4 (GPX4)	A375 melanoma cells, HeLa cervical cancer cells, 4T1 breast cancer cells, B16-F10 murine melanoma cells and SH-SY5Y human neuroblastoma cells			[74]

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E3 ubiquitin ligase complex, the MDMX binding motif, and a flexible tripolymer glycol linker (Figure 2). Due to the tumor expansion, insufficient cell permeability, and proteolytic resistance, a macromolecule called polyacryl sulfydryl imidazole (PSI) and nanoengineered gold were introduced, both of which have pH-triggered charge reversal. As anticipated, Nano-MP@PSI was able to considerably increase tumor-specific accumulation and cellular absorption by shifting the Zeta potential from 28 mV at pH 7.4 to 50 mV at pH 6.0. Furthermore, the increased glutathione content in cancer cells would cause Au(I)-alkanethiol links to break, allowing for the regulated release of MDMX-based PROTACs. As a result, the MDMX expression was significantly down-regulated in Nano-MP@PSI-treated WERI-Rb-1 cells compared with Nano-MMP@PSI, Nano-PEG@PSI, or Nano-DPMI@PSI, leading to stabilized p53, p73, and p21 (Figure 3). Accordingly, Nano-MP@PSI displayed a remarkable therapeutic response both in retinoblastoma-bearing mice and a patient-derived xenograft model, offering the possibility for a more precise delivery of PROTACs.

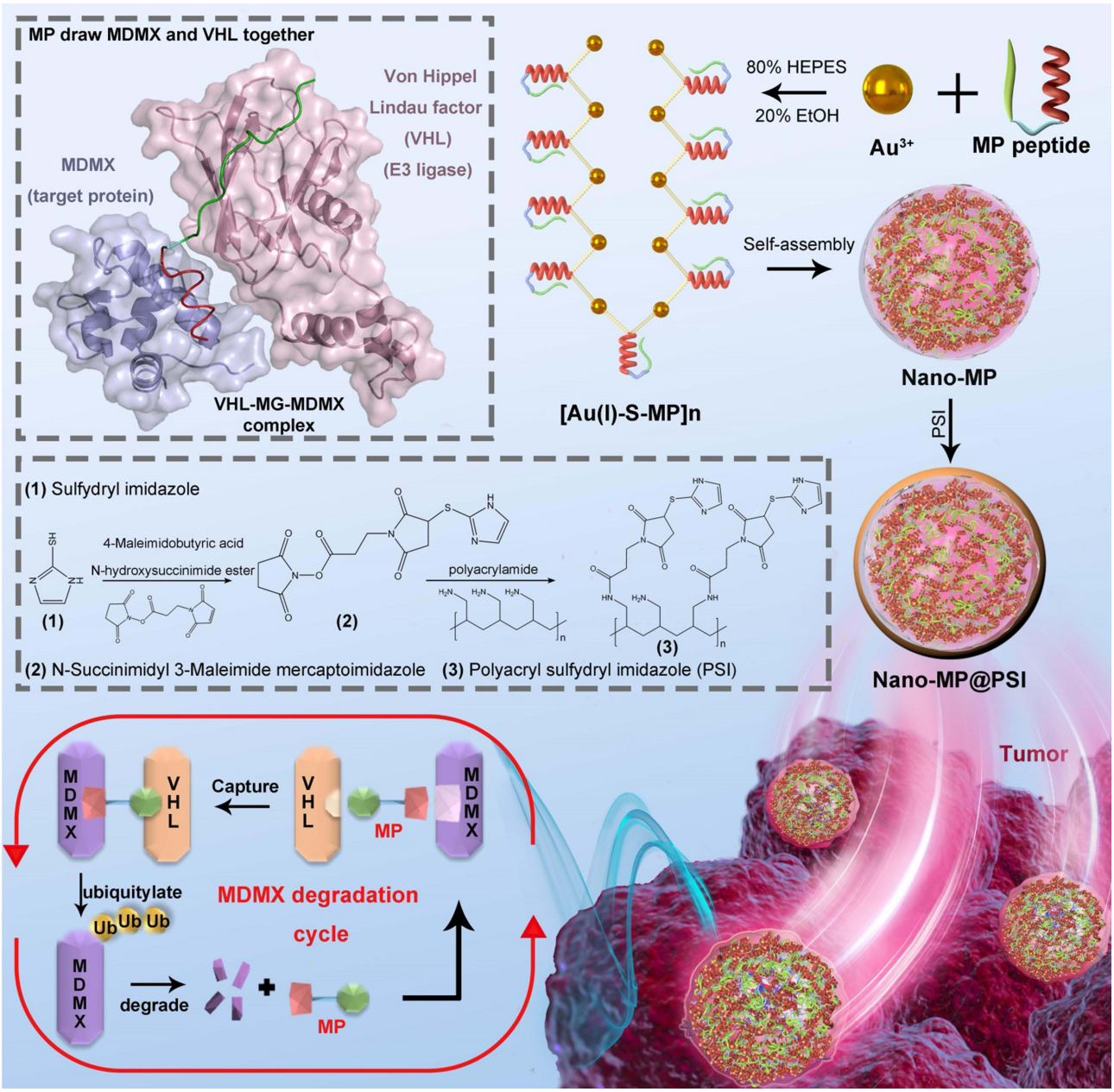

Figure 2 The MDMX degrader (MG) developed from peptides and its nanoscale approach.
Notes: Reprinted from Yan S, Yan J, Liu D, et al. A nano-predator of pathological MDMX construct by clearable supramolecular gold(I)-thiol-peptide complexes achieves safe and potent anti-tumor activity. *Theranostics*. 2021;11(14):6833–6846.[66] Creative Commons Attribution License (https://creativecommons.org/licenses/by/4.0/).

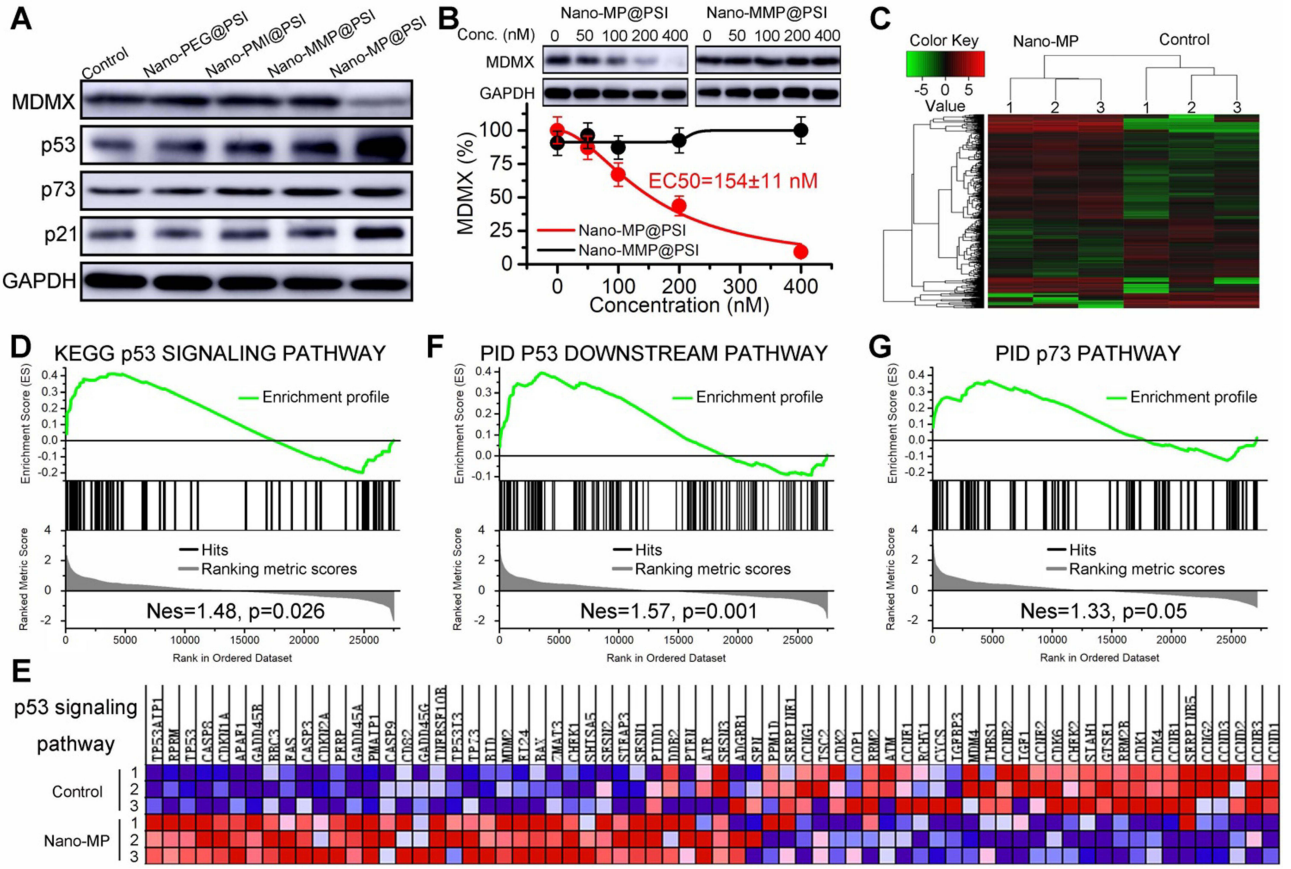

Figure 3 In WERI-Rb-1 cells, nano-MP@PSI down-regulated MDMX expression and then stabilized the expressions of p53, p73, and p21.
Notes: (**A**) Western blotting revealed the MDMX, p53, p73, and p21 protein expressions. Various preparations were added to cells and incubated for 6 hours at a dose of 100 nM. (**B**) The quantitative analysis of the Western blot (inserted photos) shows the dose-dependent degradation of MDMX. (**C**) Hierarchical clustering of genes differentially expressed in cells during a 12-hour incubation period at a dose of 1 μM with Nano-MP@PSI and no treatment. The p53 signaling pathway includes gene set enrichment analysis (GSEA, **D**) and hierarchical gene clustering (**E**). GSEA demonstrates the differential expression of the p53 (**F**) and p73 (**G**) pathways in response to Nano-MP@PSI. KEGG, Kyoto Encyclopedia of Genes and Genomes; PID, Pathway Interaction Database; NES, normalized enrichment score. Reprinted from Yan S, Yan J, Liu D, et al. A nano-predator of pathological MDMX construct by clearable supramolecular gold(I)-thiol-peptide complexes achieves safe and potent anti-tumor activity. *Theranostics*. 2021;11(14):6833–6846.[66] Creative Commons Attribution License (https://creativecommons.org/licenses/by/4.0/).

A frequent mutation in Kirsten RAS (KRAS) is detected in 95% of cases of pancreatic cancer (PC). However, because the KRAS has a roughly spherical form and no appropriate catalytic pockets, directly addressing it remains an extremely difficult process. The first-in-class PROTAC-induced PDEδ degrader (PIPD), created by Fan et al,[72] was able to effectively induce PDEδ degradation and show markedly enhanced anti-tumor activity against malignancies with KRAS mutations. However, the penetration efficiency at the PROTAC target cell membrane is poor, and untargeted delivery induces undesired "off-target" side effects. In order to solve this challenge, scientists were able to remove the PATU-8988 cells' cytomembrane, wrap PIPD molecules with ultrasonography, and create an intelligent nano-drug delivery system (CM8988-PIPD) for the targeted distribution of PIPD. Excellent serum stability, a perfectly regulated drug release profile, immunocompatibility, and biocompatibility were all present in CM8988-PIPD. Cell membrane coverage could effectively promote the endocytosis biomimetic nanosized CM8988-PIPD by homologous targeting. CM8988-PIPD had the highest apoptosis rate in all groups (53.7% in PATU-8988 cells and 54.3% in PL-45 cells). Additionally, it can downregulate p-AKT and p-Erk, effectively promote PDEδ breakdown in both concentration and time dependency, and decrease proliferation by blocking RAS signaling.

Glutathione peroxidase 4 (GPX4) is one kind of selenoprotein that could specifically catalyze lipid peroxides into lipoids, playing an important role in regulating ferroptosis. We hypothesize that inhibiting GPX4 overexpression or degradation may impair lipid peroxide elimination, which might result in cell ferroptosis.[76] To confirm this possibility,

Luo et al[74] created a PROTAC degrader that targets GPX4, and dGPX4. They achieved this by covalently coupling pomalidomide, which attracts cereblon (CRBN) E3 ubiquitin ligase, with ML162, an inhibitor of GPX4. The enhancement of intracellular ROS levels and lipid peroxidation after dGPX 4 treatment suggested that dGPX4 degradation induces ferroptosis in cancer cells. Furthermore, dGPX4 treatment caused ferroptosis in cancer cells and showed generality in various cancer cells (e g A375 melanoma cells, HeLa cervical cancer cells, 4T1 breast cancer cells, B16-F10 murine melanoma cells and SH-SY5Y human neuroblastoma cells). Because of its low biostability and poor water solubility, PROTACs limited its clinical transformation. Reactive oxygen species (ROS)-degradable lipid 401-TK-12 is used to encapsulate dGPX4 in lipid nanoparticles to get around these restrictions. Lipid nanoparticle-based dGPX4 formulation has the potential to inhibit tumor development while simultaneously improving GPX4 breakdown in tumors, all without causing noticeable adverse effects. The selective release of dGPX4 into cancer cells for the breakdown of GPX4 was caused by the upregulated intracellular ROS level, which also prompted the degradation of 401-TK-12. After 12 hours of treatment, more than 60% of the dGPX4 was thus released from the dGPX4@401-TK-12 nanoparticles, with the least amount of dGPX4 released under the same incubation conditions without H2O2.

The epigenetic reader bromodomain-containing protein 4 (BRD4) is a member of the BET bromodomain family, with two conserved bromodomains and the extra-terminal (ET) domain. Since BRD4 is often shown to be overexpressed in lung tumors, targeting BRD4 has gained popularity as a treatment strategy for the disease. To accurately destroy BRD4, Zhang et al[68] created a unique type of adaptable nano-PROTAC called CREATE. The pH/GSH-responsive polymer (DS-PLGA) loaded BRD4-targeted PROTAC (dBET6) was used to form the CREATE (CRV-LLC membrane /DS-PLGA /dBET6), which was subsequently concealed with CRV-engineered Lewis lung carcinoma (LLC) cell membranes (CRV-LLCM). The rapid breakdown of CREATE caused by the combination of low pH and high GSH concentration enhanced the therapeutic efficacy of dBET6. In contrast to the mixture containing pH-responsive but GSH-insensitive components, CREATE resulted in a notably elevated rate of apoptosis in both LLC cells and M2-like macrophages. The first TAMs-lung cancer cell spheroids (M2/LLC spheroids) were created by co-cultivating LLC cells with M2-like macrophages to assess the tumor suppressive impact in a three-dimensional tumor spheroid test. As anticipated, in comparison to DS-PLGA/DiD or LLCM/DS-PLGA/DiD, CRV-LLCM/DS-PLGA/DiD caused a much larger infiltration in the 3D M2/LLC spheroids and resulted in considerable cell death. CREATE demonstrates the capacity to target lung cancer cells and TAMs simultaneously with the use of CRV-overexpressing LLCM for camouflage. These results offer a new approach to lung cancer recurrence.

Combination Tumor Therapy

Currently, PROTAC-based immunotherapy predicts responses to a variety of malignant tumors,[70] providing a new framework for long-lasting tumor responses and augmented tumor therapeutic effects. Nevertheless, due to the inability to target particular tissues and the ensuing off-target impact, there are still a lot of obstacles and restrictions concerning low patient response rates and immune-related side effects. To enable enhanced response to immunotherapy, combination therapy can be introduced, for example between chemotherapy,[77–79] photodynamic therapy,[80] photothermal therapy,[81] and immunotherapy.[82] However, PROTACs-based off-target problems induced by nonspecific distribution remain an obstacle. To overcome this, Zhang et al[29] synthesized a semiconducting polymer nano-PROTAC (SPNpro) for photo-switchable combination therapy of immunotherapy and photodynamic therapy (Figure 4). The obtained nanodevices were composed of a semiconducting polymer backbone, a cathepsin B-cleavable peptide, and an indoleamine 2,3-dioxygenase (IDO)-targeting PROTAC. After intravenous injection, SPNpro was passively distributed into the lesion via the EPR effect. The overexpressed cathepsin B in the tumor microenvironment activated SPNpro by breaking down the cathepsin B-cleavable peptide, which facilitated the release of IDO-targeting PROTAC. PROTACs recruited E3 ubiquitin ligase and IDO, respectively, contributing to an 89.3% decreased expression of IDO, therefore altering tumor metabolism and ameliorating the tumor immunosuppressive microenvironment. The cathepsin B-switchable behavior of SPNpro rendered the local activation of PROTACs, avoiding serious off-target effects. Meanwhile, NIR irradiation activated SPNpro to generate a large quantity of $^1O_2$, exerting photodynamic therapy, followed by the release of tumor-associated antigens and immunogenic cell death. As the microenvironment was reprogrammed, more cytotoxic T lymphocytes

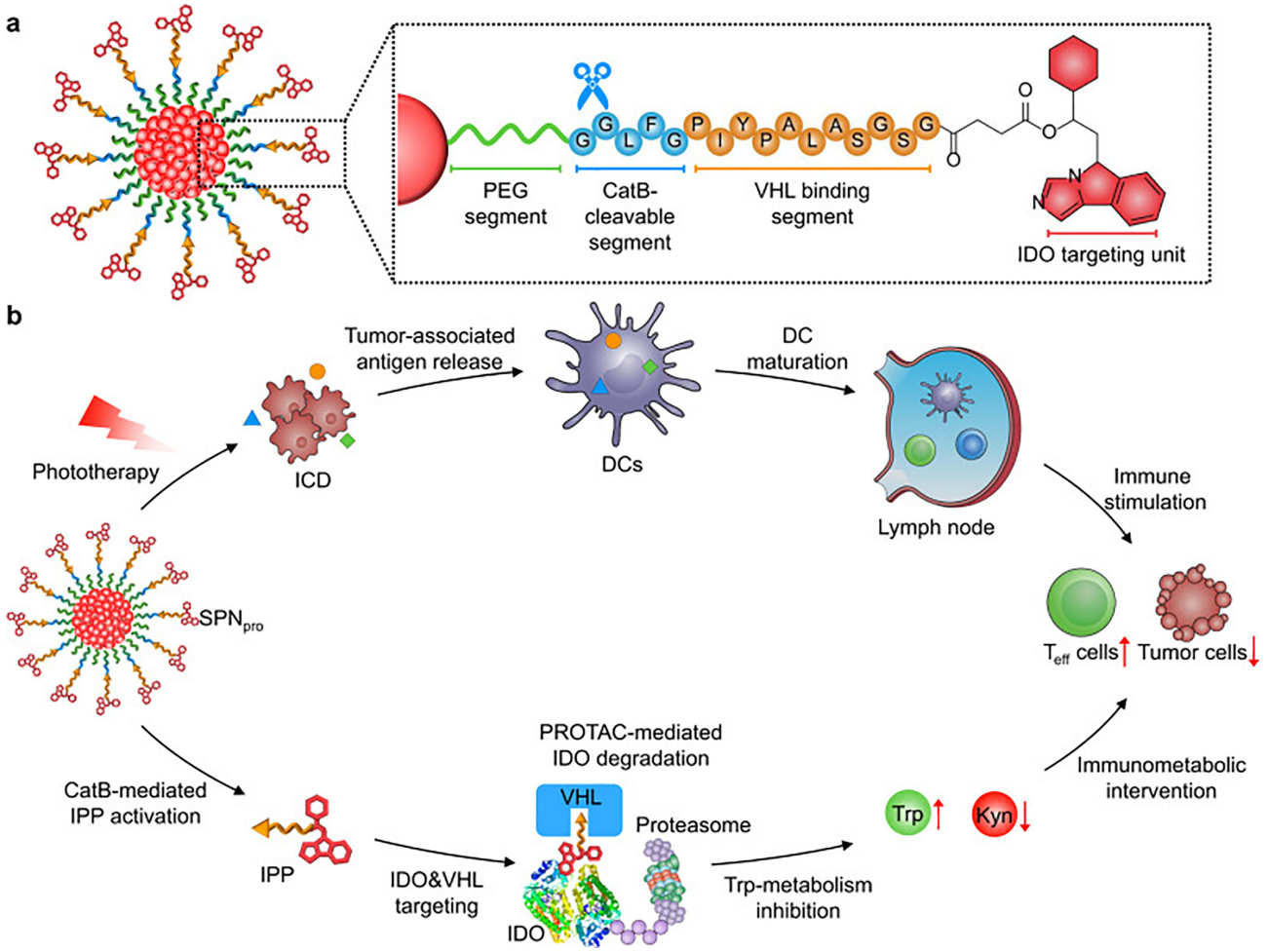

Figure 4 The antitumor mechanism of the semiconducting polymer nano-PROTAC (SPNpro).
Notes: (**a**) The structure and activation mechanism of SPNpro that is unique to cathepsin B (CatB). (**b**) Activatable photo-immunometabolic treatment mediated by SPNpro using two mechanisms. Reprinted from Zhang C, Zeng Z, Cui D, et al. Semiconducting polymer nano-PROTACs for activatable photo-immunometabolic cancer therapy. *Nat Commun*. 2021;12(1):2934.[29] Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/).

could filtrate into the tumor tissue and boost immunotherapy, realizing effective tumor growth inhibition both in primary and distant tumors without any signs of adverse effects.

Based on the same method, Zhang et al[70] synthesized another semiconducting polymer, onto which COX-1/2-targeting PROTACs were connected using a Cathepsin B-cleavable peptide. This resulted in the development of smart nano-PROTACs (SPN_{COX}), which are intended for photo-metabolic cancer immunotherapy. The diameter of this prepared nanosystem was measured at 30 nm. Under laser irradiation, SPN_{COX} indicated the potential to generate a large quantity of $^1O_2$ in a time-dependent manner. After incubation with Cathepsin B, the FK peptide sequence containing SPN_{COX} was cleaved, resulting in the rapid release of COX-1/2-targeting PROTACs followed by persistent degradation on COX-1/2. As a result, SPN_{COX} demonstrated an accelerated ability to remodel the immunosuppressive microenvironment and reinvigorate immunotherapy, thereby causing significant inhibition of tumor growths without originating tumor recurrence (Figure 5). This combination therapy of PROTACs-mediated immunotherapy and photodynamic therapy overcomes the off-target obstacles and expands our understanding of antitumor synergistic therapy.

As a new type of antitumor therapy, immune checkpoint blockade is a milestone innovation in the history of malignancy therapy. Despite these advances, it also has some problems such as inefficiency and off-target adverse effects. It is very necessary to develop an immunological checkpoint PROTAC to support the immune response against tumors. In light of this, Zhang et al[69] described a checkpoint PROTAC method that enhances the anticancer immune

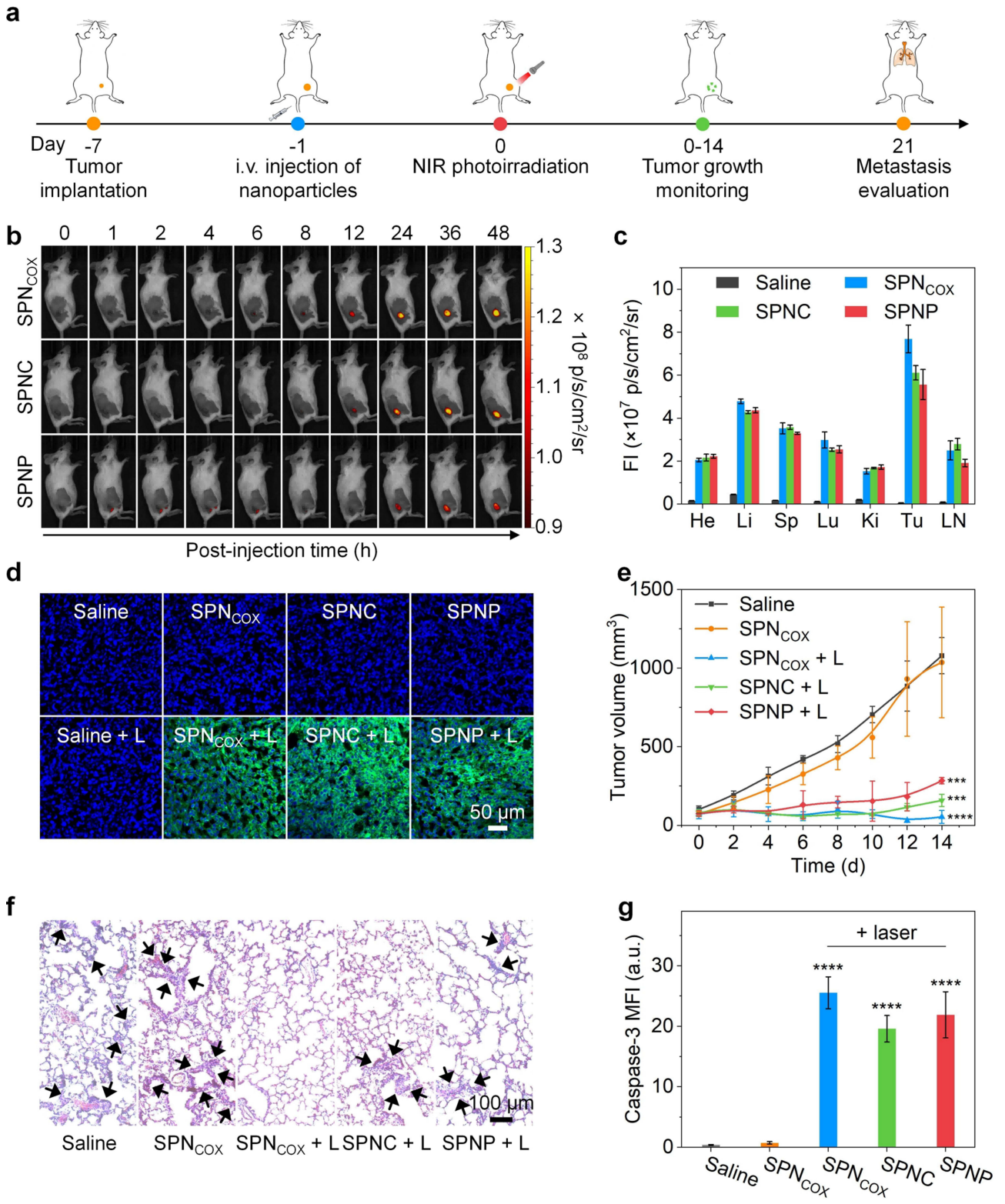

Figure 5 In vivo anti-tumor and anti-metastatic effect of SPNCOX.

Notes: (**a**) Schedule for 4T1 tumor implantation and treatment. (**b**) Biodistribution of various preparations in 4T1 tumor-bearing mice at various intervals following the intravenous injection. (**c**) A quantitative examination of Figure 4b's principal organs. (**d**) Confocal pictures of tumors treated with various preparations, either with or without 6 minutes of NIR photoirradiation (0.3 W cm−2 at 808 nm). Reactive oxygen species concentration was detected by the green fluorescent signal produced by the SOSG probe. (**e**) Tumor volume in 5 4T1 tumor-bearing mice following various treatments throughout 14 days of observation. (**f**) Lung tissues in 4T1 tumor-bearing mice stained with hematoxylin and eosin following various treatments. The metastatic nodes were shown with black arrows. (**g**) Quantitative evaluation of caspase-3 expression in tumors following various therapeutic approaches (n = 5). *** p<0.001 and **** p<0.0001. Reprinted with permission from Zhang C, He S, Zeng Z, Cheng P, Pu K. Smart Nano-PROTACs Reprogram Tumor Microenvironment for Activatable Photo-metabolic Cancer Immunotherapy. *Angew Chem Int Ed Engl*. 2022;61(8):e202114957.[70] Copyright 2022 Wiley, under the license number 5770710257931.

response by focusing on the Src homology 2 domain-containing phosphatase 2 (SHP2). This technique works in concert with immunogenic phototherapy. Photosensitizer (protoporphyrin IX, PpIX), caspase 3 lysable peptide substrate (DEVD), UBR E3 ligase targeting fragment (RLAA), and the SHP2-targeted peptide (SP, SLNpYIDLDLVK) were all part of the checkpoint nano-PROTAC (NPRO). Caspases 3 and associated with apoptosis during photodynamic therapy-induced 4T1 cell death may activate NPRO and then be transported to T cells or macrophages to cause SHP2 degradation. Because of NPRO-mediated therapy's phototherapeutic potential and strong T-cell activation through SHP2 degradation, tumor immunogenicity was increased. The immune-suppressive checkpoint signaling pathways, including PD-1/PD-L1 and CD47/SIRPα ("Don't eat me"), were suppressed by the degradation of SHP2. Following this, effector T cells (Teffs) mediated antitumor immunity and M1-type macrophages (M1Macs) mediated phagocytosis effect were triggered. Therefore, NPRO-mediated therapy's superior biosafety and biocompatibility might efficiently reverse immunosuppression to activate checkpoint signaling and strengthen immune responses against tumors.

It is well recognized that a variety of tumors and malignancies are encouraged to form, progress, and spread when the polycomb ring finger oncogene BMI1 is present. Additionally, it has been demonstrated that an unusually high BMI1 suppresses the innate immune response of tumor cells and increases resistance, especially to immunotherapy. Consequently, Wu et al[65] devised a plan to influence TME immunosuppression and concurrently lower BMI1 to enhance the clinical prognosis of patients with head and neck squamous cell cancer (HNSCC). Two types of nanoparticles were grafted onto an injectable thermosensitive nanocomposite hydrogel that they designed. The first type was coated mesoporous silica nanoparticles (MSNs) with the cancer cell membrane (CCM) and paclitaxel (Pac) in their pores, connected to a peptide-based BMI1 PROTAC drug via disulfide bonds (PepM@PacC); the second type were R837-loaded CaCO3("RC") nanoparticles. In short, thermosensitive nanocomposite hydrogel injected intratumorally was able to gel in situ but eventually broke down in TME. Following the release of PepM@PacC from the deteriorating hydrogel, cleavage of the BMI1-targeting PROTAC from the MSNs and the release of paclitaxel are facilitated by the CCM-mediated homologous targeting of cancer cells and GSH-dependent drug release. Then, in the acidic TME, the RC nanoparticles quickly dissolved and released the pre-loaded R837, which encouraged DC maturation and increased the T cell-mediated immune response even more. The PP-RC-H treatment dramatically increased the therapeutic efficacy of the tumor by reducing the tumor cells' ability to evade the immune system and inducing BMI1 degradation in addition to chemotherapy.

ARV-825 (ARV) is a form of BRD4-targeting PROTACs that holds promising effects for the treatment of vemurafenib-resistant melanoma. However, its therapeutic effect required improvements in hindering the dense extracellular matrix in tumor tissue and extremely poor aqueous solubility. Liposomes are large unilamellar vesicles consisting of phospholipids and cholesterol, which were confirmed to be available for co-delivery of both hydrophilic and hydrophobic drugs, long-circulating time[83] and enhanced therapeutic outcomes.[84] Considering the potential of liposomes, Fu et al[71] loaded BRD4-targeting PROTACs (ARV) and an anti-fibrotic agent (nintedanib) into the lipid bilayer of PEGylated liposomes for combination therapy (Figure 6). Critic acid was added during the drug loading procedure to increase the entrapment efficacy from 21.67% to over 90%. The particle size of the nano-liposome (ARNIPIL) was evaluated at 111.1 nm with a uniform size distribution. Moreover, the preparations remain stable in one month storage. An in vitro release investigation showed that ARNIPIL released the medication continuously, without experiencing any burst release. Consequently, ARNIPL significantly altered the number of colonies, vasculogenic mimicry and BRD4 expression when compared to ARV or nintedanib alone. In three-dimensional (3D) multicellular tumor spheroids, ARNIPL reduced 71.43% of tumor volume on day six. In contrast, the reduction of tumor volume with an individual drug was less than 58%. The augmented efficacy may be attributable to the enhanced penetration of liposomes. Hence, liposome-based co-delivery nanovesicles present a promising combination approach for vemurafenib-resistant melanoma therapy.

Monoclinic 1t-phase TaTe2 has remarkably paramagnetic, charge transfer, and biocompatible properties. TaTe2 can exhibit photothermal and photodynamic qualities due to the surface's charge transfer characteristics. This suggests that TaTe2 might be used as a nanotherapeutic. A nanotherapy platform (T-ARV) based on TaTe2 nanosheets loaded with ARV-825 was created by Liu et al.[73] T-ARV outperformed the conventional TMDs in terms of photostability, exhibiting a photothermal conversion of up to 62.3% and high absorption in the near-infrared spectrum. For ten minutes at 62°C, T-ARV dispersions were exposed to an NIR laser, producing singlet oxygen and superoxide anions. ARV-825 bound to a nearby E3 ligase upon coming into contact with BRD4 and T-ARVs, which started the process of BRD4 ubiquitination

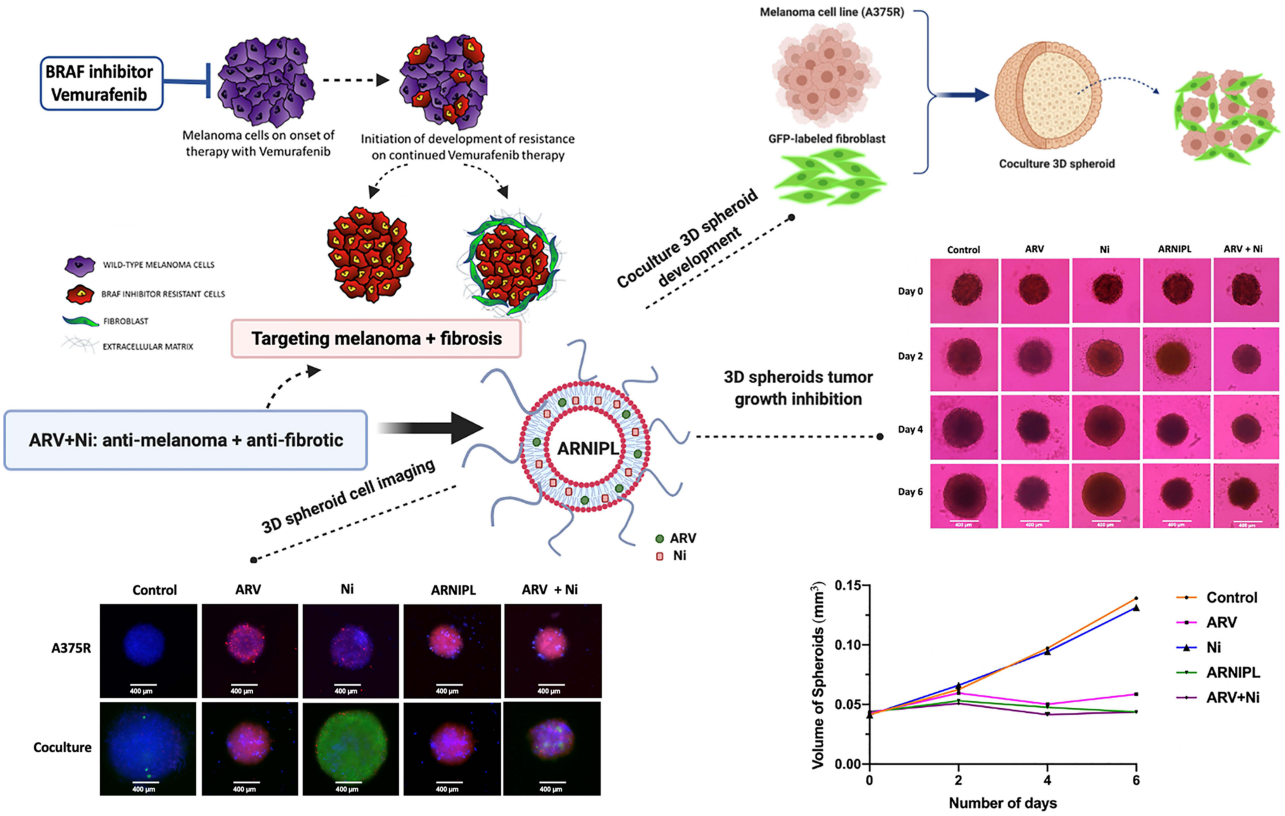

Figure 6 The preparation route and anti-tumor response of BRD4-targeting PROTACs (ARV) and an anti-fibrotic agent (nintedanib) co-loaded liposome.
Notes: Reprinted from Fu Y, Saraswat A, Wei Z, et al. Development of Dual ARV-825 and Nintedanib-Loaded PEGylated Nano-Liposomes for Synergistic Efficacy in Vemurafenib-Resistant Melanoma. *Pharmaceutics*. 2021;13(7):1005.[71] Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

and proteasomal destruction. TaTe2 nanosheets' large surface area allowed ARV-825 to attach to target proteins with efficiency, which in turn aided the proteasomal breakdown process. For a brief time, T-ARV briefly and at low concentrations broke down BRD4 and the downstream protein C-MYC. Under near-infrared light irradiation, T-ARV efficiently sensitized the photothermal and photodynamic properties of tumor tissue. Additionally, T-ARV was developed as a contrast agent for photothermal optical coherence tomography imaging (PT-OCT) used in tumor imaging. It can direct oral squamous cell carcinoma multimodal therapy.

Nano-Sized PROTAC (Nanocarriers as the Linker)

Lymphoma kinase receptor anaplastic lymphoma kinase (ALK) contributes to the growth and endurance of lung cancer epithelial cells. Thus, degradation of ALK appears to be crucial for enhanced anti-tumor effects. Several ALK-targeting PROTACs have been well explored,[72,73] showing notable advantages in specificity and efficacy. However, challenges remain in clinical applications. The chemical structure of PROTACs needs to be optimized to meet the requirements of cell penetrability and appropriate binding space. Even though optimization is finished, the prepared PROTACs still lack tissue targeting ability, which is an empirically important cause of off-target effects. Inspired by two-headed PROTACs, gold nanoparticles (GNPs) with large surface areas and versatile functionalization were excavated for the construction of multi-headed PROTAC nanoplatforms. ALK binding moiety (ceritinib) and E3 ligase ligand (pomalidomide) were grafted onto spherical GNPs (30 nm) coated with PEG2000 as a linker by Wang et al,[67] creating a nanoscale PROTAC (Cer/Pom-PEG@GNP) (Figure 7). Cer/Pom-PEG@GNP never lost its stability in the presence of serum, tumor tissue homogenate, or cell culture media, however, the particle size was slightly altered. After cellular internalization, it co-localized in the cytoplasm, providing the possibility for targeting ALK proteins. Once incubated for 12 h, 76.21% of ALK fusion proteins were degraded. As ALK protein is involved in cell proliferation, a cell viability assay

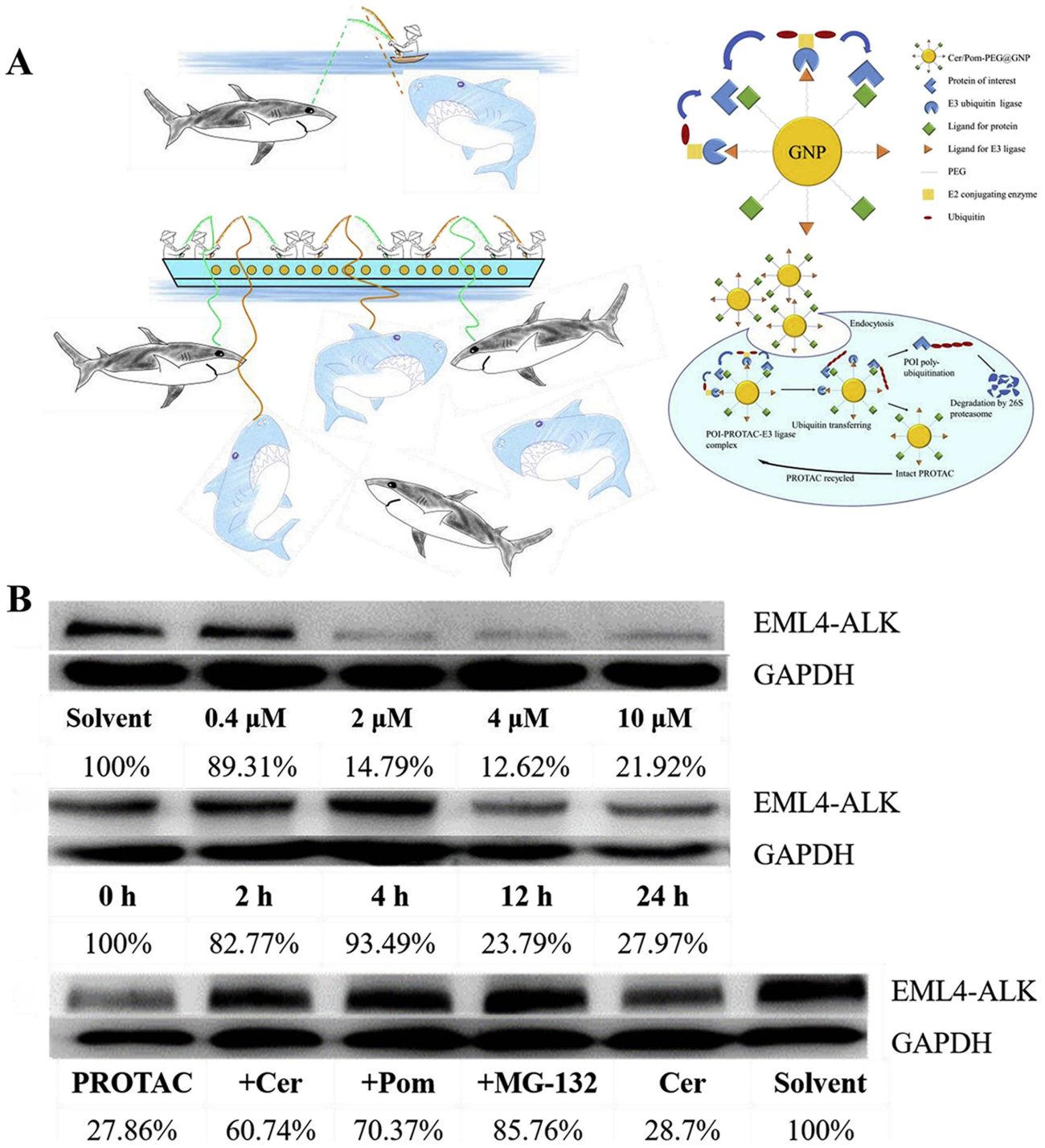

Figure 7 The schematic illustration of the nanosized PROTAC (Cer/Pom-PEG@GNP).
Notes: (**A**) The preparation process and anti-tumor mechanism of Cer/Pom-PEG@GNP. (**B**) The EML4-ALK expression was measured by Western blot under different conditions. Reprinted from *Colloids and Surfaces B: Biointerfaces*, volume 188, Wang Y, Han L, Liu F, et al. Targeted degradation of anaplastic lymphoma kinase by gold nanoparticle-based multi-headed proteolysis targeting chimeras. 110795, with permission from Elsevier.[67]

was conducted. Accordingly, the Cer/Pom-PEG@GNP nanoplatforms decreased the percentage of living cells with an IC50 of 4.8 μM, implying the potential anti-tumor response. This nanodevice presents a novel strategy to design a nano-PROTAC using nanocarriers as the linker, thereby creating a multivalent combination with target proteins and more efficient degradation.

Discussion

Despite significant achievements and advancements made using nanotechnology, nano-PROTACs still require further design. Some principles of nano-sized PROTACs or NDDS designed for PROTACs are summarized as follows:

For Delivering Existing PROTACs

Improvement in Tumor Distribution and Cellular Uptake

The particle size, morphology, and Zeta potential of nanoparticles determined their biological distribution in different organs, including the lung, liver, spleen, and kidney. Therefore, these parameters should be managed to obtain ideal NDDS delivering existing PROTACs, thereby improving tumor accumulation and cellular uptake.

Nanoparticles with a diameter larger than 2000 nm were essential to accumulate in the capillaries of the spleen, liver, and lung. Particle sizes in the range of 100–200 nm can escape the filtration of the liver and spleen and potentially accumulate in the tumor site through the EPR effect. As the size increased to more than 200 nm, more and more nanoparticles were distributed in the liver and spleen. In contrast, small-sized nanoparticles (<5 nm) were filtered out by the kidney.

Nanoparticles with different shapes, including spherical, rod, cylindrical, and disk, can be prepared by adjusting the synthesis conditions. Nanoparticles with different shapes have varying flow characteristics, cycle life, cell membrane interaction, and macrophage uptake, which in turn affect their pharmacokinetics and biodistribution among different organs.[85–87] Of all the particles under investigation, spherical forms with smaller surface areas often showed the greatest tumor-to-blood ratios and accumulation.[88] Furthermore, the in vivo dispersion of nanoparticles with varied nanostructures varies for different cells. For breast cancer cells, rods offer the best specificity and absorption efficiency.[89] In contrast, spheres performed the highest uptake in macrophages.[90]

Nanoparticles with different surface charges affect the opsonization, cycle time, and interaction with resident macrophages contained in reticuloendothelial systems.[91] Macrophages in the lung, liver, and spleen are more prone to seize positively charged particles. Neutral and slightly negatively charged nanoparticles have reduced accumulation in the reticuloendothelial system, thus, prolonging the cycle life.

Furthermore, proper surface modification of nanoparticles with targeting ligands,[92,93] antibodies,[94] or oligonucleotides[95] can potentially improve tissue accumulation. It is worth noting that membranes derived from target cells can be coated on the surface of biomaterials, providing further improvements in delivery efficiency. Similarly, extracellular vesicles were also regarded as nano-swimmers for efficient drug delivery, displaying precise tissue traffic and a homing effect.

Tumor Microenvironment-Responsive Drug Release

To avoid off-target effects, tissue-specific PROTAC release should be considered. Tumor microenvironment-responsive NDDS can serve as intelligent nanoplatforms for controlled drug release by endogenous or exogenous stimuli. Endogenous factors contain differences in tumor microenvironments from healthy tissue, including pH, enzymes, glutathione, reactive oxygen hypoxia,[96] adenosine-triphosphate,[97] and inflammatory factors.[98] PROTACs can be conjugated onto the surface of nanoplatforms with tumor microenvironment-cleavable linkers. Once they enter into tumor tissues, PROTACs-based nanodevices can be triggered to release and realize tissue-specific effects.

Unlike endogenous stimuli-responsive nanoplatforms, exogenous stimuli-triggered NDDS have the potential benefit of overcoming individual pathological differences. These technologies allow for the exact regulation of medication release with the application of external variables locally.[99] Exogenous agents refer to photo/light,[100] magnetic field,[101] ultrasound,[102] microwave,[103] mechanical forces,[104] and electric field.[105]

Various wavelengths of light, including ultraviolet, near-infrared, and visible light, have been reported and discussed. Due to their limited capacity to permeate tissues, visible and ultraviolet light are not appropriate for use in in vivo treatments. On the other hand, near-infrared spectroscopy is thought to be the best light source for controlled medication release. Near-infrared light can transform photons into heat through photothermal substances, producing a photothermal effect and splitting temperature-sensitive linkers to spatiotemporally control drug release.[106] It can also convert light into reactive oxygen species through photosensitizers to produce photodynamic effects, thereby breaking down the reactive oxygen species sensitive bonds and performing spatiotemporally controlled drug release. Furthermore, it can upconvert

nanoparticles from near-infrared light to ultraviolet or visible light, therefore releasing medicines from the backbone of UV-triggered drug release from near-infrared light.-responsive, highly monodispersity diblock copolymer-coated up-conversion nanoparticles.[107]

This is convenient to externally applied magnetic fields because magnetic field-activated materials are often used as drug release platforms.[108,109] The mechanism of controlled release mainly focuses on the abundant energy generated by the application of magnetic fields locally, which increases local temperature and breaks down the temperature-sensitive bonds.[110,111]

Similarly, ultrasound, microwave, and electric fields primarily induce local high temperatures to break the temperature-sensitive bond to produce a controlled release of drugs and reduce toxic and side effects.[112,113] In addition, ultrasound can also act on ultrasound contrast agents to create openings on the surface of NDDS to release drugs.[114] Electrochemical reactions can also alter the local pH and break the pH-sensitive bond of nanoparticles to control drug release.

Combination Therapy

Extremely aggressive tumors require combination therapy to kill them via multiple pathways. AS NDDS possess large cores with a specific surface area, they can interact with various drugs to deliver multiple drugs and realize combination therapy. Furthermore, some nanocarriers have intrinsic functions, such as photothermal conversion,[115] reactive oxygen-generating ability,[116] and neutralizing acid microenvironments,[117,118] thus, achieving synergistic effects.

Nano-Sized PROTACs

Nanocarriers can also be used as a part of PROTACs to become nano-sized PROTACs. Generally, nanocarriers are regarded as the platform to load the E3 ubiquitin ligase ligand and target protein ligand. It should be noted that the particle size of nanocarriers applied should be controlled to fulfill the formation of the ternary complex. If the size is too large, it may affect the binding efficiency and subsequent ubiquitination of the target protein and E3 enzyme due to insufficient space. Similarly, if the size is too small, it may be rapidly eliminated in the tumor site, reducing the accumulation efficiency.

Concurrently, most nanoparticles are internalized into cells via clathrin-mediated endocytosis, resulting in degradation in lysosomes rather than perforation in cytoplasm. Hence, if internalized, nanoparticles should escape from degradative endo-lysosomal compartments. Cationic nanocarriers were reported to possess the ability to escape from lysosomes. However, a positive charge that is too strong may easily form a "protein crown" during circulation at physiologic conditions, which severely affects the targeting ability. This implies that the net charge and surface charge of nanocarriers indicated a dominant position in the design of nano-sized PROTACs.

Perspect in Clinical Translation

Since its inception, PROTAC technology has garnered significant attention due to its potential to overcome the limitations associated with current cancer treatments by facilitating the degradation of target proteins.

In terms of clinical applications, Arvinas stands as the pioneering biotechnology company exclusively dedicated to the development of PROTACs.[119] Following the announcement of Phase 1 clinical trials for two PROTAC candidate drugs in 2019, numerous pharmaceutical companies have invested in the advancement of PROTACs as therapeutic agents. Arvinas LLC has successfully developed two PROTAC probes, namely ARV-110 and ARV-471, which specifically target the androgen receptor (AR) and ERα for the treatment of metastatic castration-resistant prostate cancer and metastatic ER+/HER2− breast cancer, respectively.[120,121] Numerous protein degraders, targeting a wide range of solid tumors and hematologic malignancies, have been evaluated in Phase I–III clinical trials.[122,123]

The structural elucidation of eight compounds has been completed, with seven derivatives modeled on various CRBN E3 ligase ligands the eighth being VHL PROTAC DT-2216.[124,125] In clinical evaluations, each of the seven CRBN PROTACs was administered orally, and oral bioavailability in murine models was reported for four, all exceeding 30%. In stark contrast, DT-2216 exhibited markedly low bioavailability (<0.03% in mice) and required intravenous infusion. This discrepancy in bioavailability corresponds well with predictions based on Lipinski's Rule of 5[126] and Veber's rules.[127] Generally, PROTACs are characterized by high efficacy and a catalytic mechanism of action, potentially allowing effective oral administration at low oral bioavailability or lower dosages.[128] However, this theory is contradicted by the actual dosing regimens of three CRBN

PROTACs currently in Phase II or III trials, which involve substantial daily oral doses: ARV-766 is administered in Phase II trials at doses of 100 mg and 300 mg,[129] ARV-110 in Phase II at a recommended dose of 420 mg,[130] and ARV-471 in Phase III at a recommended dose of 200 mg.[131] Notably, both ARV-110 and ARV-471 demonstrate bioavailabilities exceeding 30%, although data for ARV-766 are not yet available.

Despite the significant advantages associated with PROTAC technology, it is not without its challenges, including issues related to drug permeability, diminished bioavailability, and unintended off-target effects. Nonetheless, these technologies open expansive new avenues for biomedical research. In the realm of cancer therapy, nanomedicines have demonstrated considerable promise.[132] Nano-PROTACs, in particular, offer distinct benefits over traditional PROTAC methodologies.[133] The primary mechanism by which nanoparticles exert their effects involves augmenting intracellular permeability through pathways such as endocytosis and endosomal transport. Furthermore, leveraging the EPR effect or employing active targeting strategies to guide nanoparticles can significantly mitigate the exposure of non-target tissues to high concentrations of PROTACs. It is noteworthy that most nanomedicines, both in clinical and preclinical phases, exhibit reduced deleterious impacts on healthy tissues. Over the last decade, advances in nanomedicine have led to the development of several efficacious oral delivery systems that have improved the bioavailability of therapeutic agents, thus paving the way for the formulation of effective nano-PROTACs for cancer treatment.[134] For instance, Eudragit®, a polymer coating specifically engineered to stabilize oral drug formulations, has been shown to bolster the stability of liposomal systems for oral administration.[135] The functionalization of PLGA nanoparticles enables targeted interaction with intestinal transport proteins, enhancing drug delivery efficiency.[136] Additionally, the incorporation of polycaprolactone in the production of polymeric nanoparticles has been found to improve the oral bioavailability of ellagic acid, a potent anticancer compound with otherwise limited gastrointestinal uptake.[137] Furthermore, the clinical application of docetaxel encapsulated within the polymeric matrix BIND-014® has demonstrated profound therapeutic outcomes in treating advanced metastatic cancers and non-small cell lung cancer.[138]

An additional impediment in the realm of PROTAC design is the constrained availability of efficacious ligands for ubiquitin ligases. Although the human proteome encompasses over 600 E3 ligases,[139] the repertoire of these enzymes that are actively employed within PROTAC frameworks is limited to fewer than ten. Among these, small molecule ligands targeting VHL represent some of the earliest examples employed in PROTAC development. Conversely, CRBN molecular glue has emerged as a preferred ligand in numerous PROTAC configurations, owing to its robust degradative efficacy across a variety of substrates, its diminutive molecular size, and its relative safety profile. As delineated previously, the emergence of nanotechnology presents an innovative approach to the deployment of E3 ligases.

In summation, the application of nanotechnology to enhance the delivery and the action mechanism of PROTACs offers a viable and promising avenue for advancement.

Conclusion

The PROTAC-based targeted degradation strategy has shown significant promise in cancer treatment and is positioned to be a groundbreaking therapeutic approach. Despite its revolutionary potential in achieving precise and effective degradation of the protein of interest (POI) within the drug discovery paradigm, PROTAC still faces limitations that hinder its clinical application. These limitations include off-target effects, poor cell membrane permeability, and the hook effect. The emergence of nanotechnology provides a promising opportunity to address these challenges associated with traditional PROTACs. Previous studies have discussed successful implementations of nanotechnology in this area.[29,72,74] Notably, Zhang et al[140] reported intracellular fabricated nano-PROTACs that employ self-assembling peptides as carriers for linking the POI and E3 ligase. Through the formation of polyvalent POI cellular nanofibrils, effective dose-dependent protein degradation is achieved in vivo, leading to the inhibition of tumor growth. This innovative approach successfully overcomes the off-target side effects and "hook effect" associated with traditional PROTACs.

The application of nanoplatforms for the effective delivery of PROTACs may yield improvements in biodistribution, membrane permeability, and controlled PROTAC release, thereby fundamentally enhancing the precise degradation of target proteins and enabling more effective treatment. Furthermore, the utilization of intelligent drug delivery systems can mitigate off-target toxicity risks by improving drug solubility, membrane permeability, and cell targeting. While current research efforts have primarily concentrated on evaluating the anti-tumor efficacy of nano-PROTACs, it is

important to recognize that addressing potential safety risks through appropriate preclinical models is essential for the successful clinical translation of any promising new drug modality. Therefore, conducting a comprehensive safety assessment and thorough research on nano-PROTACs is crucial.

In Conclusion, the emerging nano-PROTAC drug modality holds great promise for tumor therapy and potentially other diseases. However, careful attention must be given to key safety risks and issues throughout the clinical translation.

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Disclosure

The authors report no conflicts of interest in this work.

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